PATENT
Docket No.: **22221/1180 (RU 339CIP)**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	O'Donnell et al.	Examiner: R. Hutson
Serial No.	:	10/673,127	Art Unit: 1652
Cnfrm. No.	:	1311	
Filed	:	September 26, 2003	
For	:	BACILLUS STEAROTHERMOPHILUS BETA POLYMERASE SUBUNIT AND USE THEREOF	

AMENDMENT

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

In response to the office action dated March 22, 2006, please amend the above-identified application as follows:

Amendments to the Specification appear beginning on page 2 of this paper.

Amendments to the Claims begin on page 3 of this paper.

Remarks begin on page 5 of this paper.

Amendments to the Specification

Please replace the paragraph appearing at page 13, lines 1-3 with the following amended paragraph:

FIGURE 4C depicts the isolated DNA coding sequence for the *dnaX* gene (also present in FIGURES 4A and 4B ~~3A and 3B~~) in accordance with the invention, which corresponds to SEQ. ID. No. 3.

Please replace the paragraph appearing at page 75, lines 7-31 with the following amended paragraph:

The XbaI insert encoded an open reading frame, starting with a GTG codon, of 529 amino acids in length (58.0 kDa), closer to the predicted length of the *B. subtilis* τ subunit (563 amino acids, 62.7 kDa mass)(Alonso et al., 1990) than the *E. coli* τ subunit (71.1 kDa)(Yin et al., 1986). The *dnaX* gene encoding the γ/τ subunits of *E. coli* DNA polymerase III holoenzyme is homologous to the *holB* gene encoding the δ' subunit of the γ complex clamp loader, and this homology extends to all 5 subunits of the eukaryotic RFC clamp loader as well as the bacteriophage gene protein 44 of the gp44/62 clamp loading complex (O'Donnell et al., 1993). These gene products show greatest homology over the N-terminal 166 amino acid residues (of *E. coli dnaX*); the C-terminal regions are more divergent. ~~Fig. 4 shows~~ Figures 5A-B show an alignment of the amino acid sequence of the N-terminal regions of the *T.th. dnaX* gene product to those of several other bacteria. The consensus GXXGXGKT (SEQ. ID. No. 17) motif for nucleotide binding is conserved in all these protein products. Further, the *E. coli* δ' crystal structure reveals one atom of zinc coordinated to four Cys residues (Guenther, 1996). These four Cys residues are conserved in the *E. coli dnaX* gene, and the γ and τ subunits encoded by *E. coli dnaX* bind one atom of zinc. These Cys residues are also conserved in *T.th. dnaX* (shown in ~~Fig. 4~~ Figures 5A-B). Overall, the level of amino acid identity relative to *E. coli dnaX* in the N-terminal 165 residues of *T.th. dnaX* is 53 %. The *T.th. dnaX* gene is just as homologous to the *B. subtilis dnaX* (53 % identity) gene relative to *E. coli dnaX*. After this region of homology, the C-terminal region of *T.th. dnaX* shares 26% and 20% identity to *E. coli* and *B. subtilis dnaX*, respectively. A proline rich region, downstream of the conserved region, is also present in *T.th. dnaX* (residues 346-375), but not in the *B. subtilis dnaX* (see Figs. 3A and 3B). The overall identity between *E. coli dnaX* and *T.th. dnaX* over the entire gene is 34%. Identity of *T.th. dnaX* to *B. subtilis dnaX* over the entire gene is 28%.

Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application.

1. (Currently amended) An isolated *Bacillus* beta subunit of a DNA polymerase III-type enzyme, the isolated beta subunit~~:~~

~~(i) comprising the amino acid sequence of SEQ ID NO: 174; or~~

~~(ii)~~ being encoded by a nucleic acid molecule hybridizing to the complete complement of SEQ ID NO: 173 under hybridization conditions that are at least as stringent as use of a medium comprising ~~at most about~~ 0.9M sodium citrate buffer at a temperature of ~~at least about~~ 37°C.

2. (Original) The isolated *Bacillus* beta subunit according to claim 1 wherein the *Bacillus* species is *Bacillus stearothermophilus*.

3-4 (Cancelled)

5. (Original) The isolated *Bacillus* beta subunit according to claim 1 wherein the beta subunit is purified.

6. (Original) A beta clamp comprising the *Bacillus* beta subunit according to claim 1.

7. (Original) A DNA polymerase III-type enzyme complex comprising the beta clamp according to claim 6.

8. (Original) A kit comprising:

a container that contains therein either a deoxynucleoside triphosphate or a dideoxynucleoside triphosphate; and

a container that contains therein the DNA polymerase III-type enzyme complex according to claim 7.

9. (New) The isolated *Bacillus* beta subunit according to claim 1, wherein the hybridization conditions comprise a medium comprising 20% formamide and 0.9M sodium citrate buffer and at a temperature of 42°C, followed by washing in 0.2X sodium citrate buffer at 42°C.

10. (New) The isolated *Bacillus* beta subunit according to claim 1, wherein the hybridization conditions comprise a medium comprising 5X sodium citrate buffer and at a temperature of 65°C, followed by washing in 5X sodium citrate buffer at 65°C.

11. (New) The isolated *Bacillus* beta subunit according to claim 1, wherein the beta subunit encoded by the nucleic acid molecule is at least 80 percent identical to the amino acid sequence of SEQ ID NO: 174.

12. (New) The isolated *Bacillus* beta subunit according to claim 1, wherein the beta subunit encoded by the nucleic acid molecule is at least 90 percent identical to the amino acid sequence of SEQ ID NO: 174.

13. (New) The isolated *Bacillus* beta subunit according to claim 1, wherein the beta subunit encoded by the nucleic acid molecule is at least 95 percent identical to the amino acid sequence of SEQ ID NO: 174.

14. (New) The isolated *Bacillus* beta subunit according to claim 1, wherein the nucleic acid molecule is at least 90 percent identical to the nucleotide sequence of SEQ ID NO: 173.

15. (New) The isolated *Bacillus* beta subunit according to claim 1, wherein the nucleic acid molecule is at least 95 percent identical to the nucleotide sequence of SEQ ID NO: 173.

16. (New) An isolated beta subunit comprising the amino acid sequence of SEQ ID NO: 174.

17. (New) A beta clamp comprising the beta subunit according to claim 16.

18. (New) A DNA polymerase III-type enzyme complex comprising the beta clamp according to claim 17.

19. (New) A kit comprising:

a container that contains therein either a deoxynucleoside triphosphate or a dideoxynucleoside triphosphate; and

a container that contains therein the DNA polymerase III-type enzyme complex according to claim 18.

Remarks

In view of the above amendments and the following remarks, reconsideration of the outstanding office action is respectfully requested.

Claim 1 has been amended, claims 3 and 4 have been cancelled without prejudice, and new claims 9-19 have been added. Descriptive support for new claims 9 and 10 appears in the first full paragraph on page 30 and the third full paragraph on page 35, respectively; descriptive support for new claims 11-13 appears in the first full paragraph on page 34; and descriptive support for new claims 14 and 15 appears in the first full paragraph on page 30. New claim 16 finds descriptive support in original claim 3 (i.e., claim written in independent form), and new claims 17-19 find descriptive support in original claims 3 and 6-8. Claims 1, 2, and 5-19 are pending.

The objection to the specification is overcome by the above amendments. Although applicants disagree with the assertion made by the U.S. Patent and Trademark ("PTO"), the present claim language is clearly supported by the first full paragraph on page 30, along with the disclosure of the nucleic acid sequence of SEQ ID NO: 173 and the corresponding amino acid sequence of SEQ ID NO: 174.

The objections to claims 1, 3 and 4 are overcome by the above amendments and should be withdrawn.

The rejection of claim 5 under 35 U.S.C. §112 (second paragraph) for indefiniteness is respectfully traversed. The PTO has taken the position that the term "purified" is unclear in view of the language "isolated" as used in claim 1. Applicants respectfully disagree.

The term "isolated" connotes that the claimed beta subunit is in an environment that is distinct from that of the native beta subunit, i.e., the protein no longer exists in a cellular environment. In contrast to a beta subunit that can exist, for example, in a protein extract obtained from a cell, a purified beta subunit is one that is substantially separated from other proteins. Both isolated beta subunit and purified beta subunit are described in the procedure recited in Example 22 for the recombinant expression of *A. aeolicus* beta subunit. In particular, Example 22 describes cell lysate containing the recombinant beta subunit (i.e., isolated but not yet purified protein), as well as the purification of beta subunit from the cell lysate (first via Sephacel column and then via

Heparin Agarose column). Thus, "purified" and "isolated" are distinct terms, and persons of skill in the art would understand the distinction between these two terms.

For these reasons, the rejection of claim 5 is improper and should be withdrawn.

The rejection of claims 1, 2, and 4-8 under 35 U.S.C. §112 (first paragraph) as lacking written descriptive support is respectfully traversed.

The burden of establishing that an application lacks adequate written descriptive support falls on the PTO. *See In re Wertheim*, 541 F.2d 257, 263, 191 USPQ 90, 97 (CCPA 1976) ("[T]he PTO has the initial burden of presenting evidence or reasons why persons skilled in the art would not recognize in the disclosure a description of the invention defined by the claims."). Hence, the PTO must demonstrate *why* the disclosure is insufficient.

The Federal Circuit has clearly espoused that *per se* conclusions of written description violations cannot be founded upon the basis of genus size alone. *See Enzo Biochem, Inc. v. Gen-Probe Inc.*, 296 F.3d 1316, 1326-27, 63 USPQ2d 1609, 1614-15 (Fed. Cir. 2002) (refusing to adopt position that three species as a matter of law cannot satisfy written description requirement for significantly larger genus). Thus, the PTO's conclusion cannot be based on genus size alone. But that is precisely what the PTO has done at page 4 of the outstanding office action. Because the PTO's position is unsupported by law and unsupported by any facts other than genus size, applicants submit that the PTO's position cannot be sustained.

In contrast, applicants present Exhibits 1-3 (attached hereto) as evidence that the nucleic acid sequence of SEQ ID NO: 173 and the corresponding amino acid sequence of SEQ ID NO: 174 represent the claimed genus. Exhibit 1 is a presentation of Genbank accessions for *Bacillus* or *Geobacillus* (formerly *Bacillus*) *dnaN* nucleic acids that are homologous to the nucleotide sequence of SEQ ID NO: 173. These *dnaN* nucleic acids were identified by a protein-protein BLAST search of the Genbank database performed using the amino acid sequence of SEQ ID NO: 174 and the BLAST default settings. Homologous sequences were identified in *Geobacillus kaustophilus, Bacillus cereus*, *Bacillus thuringiensis*, *Bacillus weihenstephanensis*, *Bacillus subtilis*, and *Bacillus licheniformis* (Exhibit 1). Based upon alignments performed using Align® for nucleic acids and ClustalW for amino acids (using the European Molecular Biology Laboratory server and its default settings), these homologs share between about 55 and about 87 percent identity at the nucleic acid level (Exhibit 2) and between about 69 and about 97 percent identity at the amino acid

level (Exhibit 3). Thus, species of beta subnits from organisms that belong to the biological classification *Bacillus* or *Geobacillus* clearly share similar structure and, therefore, function.

Applicants submit that the language recited in claim 1 is precisely the type of claim language that was acknowledged in *Univ. of California v. Eli Lilly*, 119 F.3d 1559, 43 USPQ2d 1398 (Fed. Cir. 1997) as being acceptable under the written description requirement. In *Eli Lilly*, the Federal Circuit addressed the validity of several claims of U.S. Patent No. 4,652,525 to Rutter et al. ("Rutter"), specifically those claims that recited the limitations 'vertebrate,' 'mammalian,' or 'human' cDNA for insulin. Rutter disclosed the nucleotide and amino acid sequences of a rat cDNA encoding insulin, but merely described a general procedure for obtaining the human cDNA encoding insulin. *Id.* at 1567, 43 USPQ2d at 1405. The Federal Circuit found that the description of the rat cDNA did not provide adequate descriptive support for the narrow subgenus of 'human' cDNA (no species disclosed), the larger subgenus of 'mammalian' cDNA (only the one rat species disclosed), and the larger genus of 'vertebrate' cDNA (only the one rat species disclosed). *Id.* at 1567-68, 43 USPQ2d at 1405. The Federal Circuit did acknowledge, however, the district court's statement that the specification provided adequate written descriptive support for the subgenus of 'rat' cDNA encoding insulin. *Id.* at 1566.

Thus, functional language should be acceptable when the genus as claimed is sufficiently limited in scope (i.e., from *Bacillus*, including *Geobacillus*, or *Bacillus stearothermophilus*) and the specification describes one or more species within that genus. Claim 1 recites the same type of functional claim language that was identified as acceptable in *Eli Lilly* given the description of a single species by its nucleotide sequence. Thus, it should be evident that claim 1 (and claims dependent thereon) find written descriptive support in the present application.

It should be noted that the "Guidelines for Examination of Patent Applications Under the 35 U.S.C. 112 ¶ 1, 'Written Description' Requirement," make explicitly clear that the description of a representative number of species does *not* require the description to be of such a nature that it would provide support for each species that the genus embraces. 66 Fed. Reg. 1099, 1106 (2001). Hence, the absence of sequences for the later-identified *dnaN* and beta homologs is irrelevant to the issue of whether the present specification provides adequate written descriptive support for their use in accordance with the present invention.

Moreover, the conclusion by the PTO is contrary to evidence submitted herewith by applicants. As demonstrated by Exhibits 1-3, one of ordinary skill in the art would have understood that applicants were in possession of the presently claimed invention

at the time the present application was filed. This is so, because persons of skill in the art would have expected sufficiently related organisms from the genus *Bacillus* (and now *Geobacillus*) to possess homologous *dnaN* nucleotide sequences or beta proteins. Exhibits 1-3 confirm this expectation to have been reasonable.

In view of all of the foregoing, applicants submit that the rejection of claims 1, 2, and 4-8 is improper and should be withdrawn.

The rejection of claims 1, 2, and 4-8 under 35 U.S.C. §112 (first paragraph) for lack of enablement is respectfully traversed.

It is the position of the PTO that the specification does not provide sufficient guidance for making and using other beta subunit proteins within the scope of the claims. Applicants respectfully disagree.

The PTO is respectfully reminded that all that is needed is objective enablement of what is claimed. *In re Wright*, 999 F.2d 1557, 1561, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993). The present application provides the nucleotide sequence of *Bacillus stearothermophilus dnaN* (e.g., SEQ ID NO: 173) and describes how one of ordinary skill can isolate homologs of the disclosed sequence (*see* page 41, line 9 to page 42, line 29; Example 12), express the beta subunit encoded by such homologous *dnaN* sequences (*see* Examples 12 and 22), and test the encoded beta subunit for activity (*see* Examples 26 and 30, using *Aquifex* beta subunit in assay). Thus, one of ordinary skill in the art would have been fully able to make and use DNA molecules and the encoded beta subunits within the scope of the presently claimed invention.

Moreover, with regard to method 3 for homolog identification, described at page 42, that is precisely the approach used to identify the *dnaN* homologs shown in Exhibit 1 (i.e., from other *Bacillus* or *Geobacillus* organisms). For this reason, it should be apparent that the present application fully enables the production and use of other species of *Bacillus or Bacillus (*now *Geobacillus*) *stearothermophilus* beta subunit homologs.

In view of all of the foregoing, applicants submit that the rejection of claims 1, 2, and 4-8 for lack of enablement is improper and should be withdrawn.

Because claim 1 is allowable for the reasons noted above, applicants further submit that new claims 9-15 also are allowable. Moreover, because claim 3 was not rejected and has been written in independent form as new claim 16, applicants submit that claims 16-19 also are allowable.

In view of all of the foregoing, applicant submits that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

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